

Claims

1. A method of obtaining a nucleic acid encoding a proteinaceous binding domain that binds a target material comprising: <sup>1AA</sup> <sup>SP</sup>

- 5 a) providing a variegated population of eukaryotic cells, <sup>SP</sup> each cell including a nucleic acid construct coding for a potential binding protein, each said construct comprising DNA encoding (i) a potential binding domain
- 10 having one or more variable residue positions and one or more non-variable residue positions, and (ii) an outer surface transport signal for obtaining the display of the potential binding domain on the outer surface of the cell, wherein said variegated population
- 15 of cells collectively display a plurality of different potential binding domains, the differentiation among said potential binding domains, occurring as a result of amino acid variation at said one or more variable residue positions,
- 20 (b) causing the expression of said potential proteins and the display of said potential binding domains on the outer surface of said cells;
- (c) contacting said cells with the predetermined target material such that said potential binding
- 25 domains and the target material may interact;
- (d) separating cells displaying a potential binding domain that binds the target material from cells that do not so bind, and
- (e) recovering at least one cell displaying on its
- 30 outer surface binding protein comprising a successful binding domain (SBD) which bound said target, said cell enclosing SBD-encoding nucleic acid, and amplifying said SBD-encoding nucleic acid in vivo or in vitro.
- 35 2. The method of claim 1 where one or more of said binding domains comprise an amino acid sequence which is at least 88% identical with the variable region of a naturally occurring immunoglobulin heavy chain.

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3. The method of claim 1 where one or more of said binding domains comprise an amino acid sequence which is at least 88% identical with the variable region of a naturally occurring immunoglobulin light chain.

5 4. The method of claim 1 where said binding protein comprises a single chain antibody.

5. The method of claim 1 where one or more of said binding domains consists essentially of an amino acid sequence which is at least 88% identical with the variable  
10 region of a naturally occurring immunoglobulin heavy chain.

6. The method of claim 1 where one or more of said binding domains consists essentially of an amino acid sequence which is at least 88% identical with the variable region of a naturally occurring immunoglobulin light chain.

15 7. The method of claim 1 where said binding protein consists essentially of a single chain antibody.

8. The method of claim 1 where said binding protein comprises an Fab fragment of a naturally occurring antibody.

20 9. The method of claim 1 where said binding protein consists essentially of an Fab fragment of a naturally occurring antibody.

10. The method of claim 2 in which one or more variable residues correspond to residues in a hypervariable region of a naturally occurring immunoglobulin heavy chain.

25 11. The method of claim 3 in which one or more variable residues correspond to residues in a hypervariable region of a naturally occurring immunoglobulin light chain.

12. The method of claim 5 in which all of the variable residues correspond to residues in a hypervariable region of  
30 a naturally occurring immunoglobulin heavy chain.

13. The method of claim 6 in which all of the variable residues correspond to residues in a hypervariable region of a naturally occurring immunoglobulin light chain.

35 14. The method of claim 1 wherein, in said step (a), the differentiation among said potential binding domains is limited to no more than 20 amino acid residue positions of said domains.

15. The method of claim 1 wherein said potential

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binding domains are all at least 30% identical in amino acid sequence to each other.

16. The method of claim 1 in which said population of eukaryotic cells is obtained by subcloning a mixture of DNA encoding a plurality of different proteins, comprising different potential binding domains.

17. The method of claim 1 in which said mixture of DNA is synthesized, without a template, by nonbiological means.

18. The method of claim 1 in which the potential binding domains of said mixed population differ at positions predetermined prior to synthesis of said mixture of DNA.

19. The method of claim 1 in which the outer surface transport signal is an outer surface protein of said cell or a portion thereof functional to direct said display.

20. A method of producing a binding protein which binds a predetermined target material which comprises (i) obtaining, by the method of claim 1, a first nucleic acid construct encoding a binding protein having a binding domain which binds the predetermined target material, and (ii) producing either said chimeric binding protein or a second binding protein comprising essentially the same binding domain.

21. The method of claim 20 in which the target is an enzyme.

22. The method of claim 21 in which the target is a serine protease.

23. The method of claim 22 in which the target is human neutrophil elastase.

24. The method of claim 1 in which the binding domain is at least 30% identical to bovine pancreatic trypsin inhibitor.

25. The method of claim 24 in which the target is human neutrophil elastase.